Biocatalysis and Agricultural Biotechnology 19 (2019) 101097

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Nitrate (NO_3^-) and phosphate (PO_4^{3-}) removal from aqueous solutions by microalgae *Dunaliella salina*

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ARTICLE INFO

Keywords:
Dunaliella salina
Adsorption
Nitrate and phosphate ions

ABSTRACT

This study was conducted to investigate the mechanism involved in the removal of nitrate (NO_3^-) and phosphate (PO_4^{3-}) by microalgae, *Dunaliella salina* from aqueous solutions through a series of batch experiments and FT-IR analyses. The effect of various operation parameters including pH of the solution, microalgae dosage, initial NO_3^- and PO_4^{3-} concentrations and interference of other cations and anions on the uptake capacity and removal efficiency of *D. salina* was evaluated. The NO_3^- and PO_4^{3-} concentrations were measured with spectrophotometric methods. The results showed that the maximum adsorption of NO_3^- and PO_4^{3} with using *D. salina* as adsorbent were 332 and 544 mg g^{-1} and the best removal efficiency ofNO_3^- and PO_4^{3} were obtained 54% and 82%, respectively. These results were obtained at pH 7, 0.05 g L^{-1} biomass dosage of microalgae and 350 mg L^{-1} of initial NO_3^- and PO_4^{3-} concentrations. The experiments of reusing adsorbent material thought adsorption-desorption cycling showed that the reuse ability of this algae for phosphate desorption is higher than nitrate. Also, the removal efficiency and uptake capacity were decreased in the presence of other interfering anions and cations, except for NaCl, which showed a positive effect on the uptake capacity of phosphorus by algae. The results of this investigation suggested that *D. salina* biomass is suitable as an adsorbent material for recovery and adsorption of $NO_3^-$and PO_4^{3-}ions from aqueous solutions.

1. Introduction

Nitrogen and phosphorus nutrients are natural component of aquatic ecosystems and proper amounts of these nutrients are needed for normal functioning of living organisms of aquatic ecosystems (Lodi et al., 2003; Mohseni-Bandpi et al., 2013; Rasoul-Amini et al., 2014). However, the excessive content of Nitrate (NO_3^-) and Phosphate (PO_4^{3-}) in surface and ground waters is especially troublesome these days. High discharge of these nutrients in water could cause eutrophication, which disturbs the ecological balance of organisms present in water. In addition, the consumption of drinking water high in nitrates causes methemoglobinemia in infants, liver damage and carcinogenic diseases (Knobeloch et al., 2000; Fewtrell, 2004; He et al., 2018).

The major sources of NO_3^- and PO_4^{3-} come from human activities, primarily through the addition of fertilizers to crops, lawns and gardens, from municipal and home sewage systems and animal feed. Unfortunately, the eighty percent of municipal wastewater is discharged into water bodies untreated each year (Costa et al., 2004; Connor et al., 2017). The World Health Organization (WHO) and US Environmental Protection Agency (EPA) have set safety limits for the allowable concentration of nitrate for no more than 50 and 10 mgL^{-1} respectively in drinking waters and also for phosphate 1 mgL^{-1} is mentioned in drinking waters (Yang et al., 2010; Taziki et al., 2015). Therefore, effective removal of excess nitrate and phosphate from water is critical to prevent eutrophication and restore water quality (Kumar and Saramma, 2012). Today, several types of treatment processes include chemical precipitation, reverse osmosis and biological treatment are applied for removal of these nutrients from water (Sayadi et al., 2016; Rashid et al., 2017) Recently, algae based biological treatment has gained great interest because of its high efficiency, minimum cost, easy and simple operation and applicability at lower concentrations (Sabeti et al., 2018). Lau et al. (1998) found that the removal efficiency of microalgae, *Chlorella vulgaris* was 86% and 70% for nitrogen and phosphorus removal, respectively (Lau et al., 1998). Also, the cyanobacterium, *Spirulina platensis* was used to verify the possibility of employing microalgae biomass to reduce the contents of nitrate and phosphate in wastewaters and results showed that *S. platensis* would be effective for nitrate removal only at relatively low concentrations (Lodi et al., 2003). Also, Kumar and Saramma (2012) suggest that

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https://doi.org/10.1016/j.bcab.2019.101097
Received 24 January 2019; Received in revised form 13 March 2019; Accepted 14 March 2019
Available online 18 March 2019
1878-8181/

cyanobacterium *Gloeocapsa gelatinosa* is a promising biological agent for nutrient removal from wastewater (Kumar and Saramma, 2012). The mechanism of binding ions by dried algal biomass may depend on the species and the ionic charges, the algal organism, and other external environmental factors such as pH, ions concentration, biomass dosage, and temperature (Khani et al., 2008).

The aim of present study was investigate the NO_3^- and PO_4^{3-} uptake capacity and removal efficiency of the wastewater with unicellular green microalgae, *D. salina.* Some factors including pH of solution, biomass dosage of microalgae and initial nitrate and phosphate concentrations were studies in batch systems. FTIR experiments are also designed to examine how ions are absorbed by algae. In addition, the desorption experiments and reuse of algae, as well as the effect of the presence of anions and cations on the adsorption process were investigated.

2. Material and method

2.1. Preparation of biosorbent

The dry powder of the unicellular algae *D. salina*, used (as biosorbent) in this study, was obtained from Iranian Fisheries Science Research Institute (IFSRI), Offshore Research Center, Chabahar, Iran. *D. salina* was grown at 30 °C in aerated liquid media. The F/2 media was used for microalgae culture. The pH of the feed media of *D. salina* was adjusted to 7.0 by addition of 1 M H_2SO_4 and 1 M NaOH. The media was illuminated with a light/dark cycle of 16/8 h. The algal cells at the exponential phase were harvested by centrifugation at 7,000 rpm for 20 min at 4 °C. The biomass was then washed twice with distilled water. Later, it was freeze-dried for 24 h, sieved through a 100-size mesh, and stored in a desiccator until further use.

2.2. Preparation of NO_3^- and PO_4^{3-} stock solutions

The NO_3^- and PO_4^{3-} nutrient stock solution were prepared by diluting the corresponding salts namely, KNO_3 and K_2HPO_4 in distilled water. The stock solutions were diluted with distilled water for the preparation of working solution. Different initial concentration (50, 100, 150, 200, 250, 300, 350 mgL^{-1}) of working solutions of nitrate and phosphate were prepared. 100 ml of nutrient stock solution were taken in 250 ml Erlenmeyer flasks. At the start of the experiment, pH of each test solution was adjusted to the required level with diluted H_2SO_4 (1M) or NaOH (1M) solutions.

2.3. NO_3^- and PO_4^{3-} ions adsorption studies

The adsorption of NO_3^- and PO_4^{3-} ions from aqueous solutions were studied in batch systems. The effect of various operation parameters including pH of the solution, microalgae dosage, initial NO_3^- and PO_4^{3-} concentrations and interference of other cations and anions on the uptake capacity and removal efficiency of *D. salina* were studied. The effects of these factors were investigated at NO_3^- and PO_4^{3-} ions concentrations of 50–350 mgL^{-1}, with biomass dosage of 0.05–2 gL^{-1}, in the pH range of 2.0–8.0 (adjusted with H_2SO_4 or NaOH at the beginning of the experiment and not controlled afterwards) and in the present of other interference cations and anions including, $AgSO_4$, $MgSO_4$, K_2SO_4, NaCl, $NaSO_4$, $NaCO_3$. The experiments were conducted in 250-mL Erlenmeyer flasks, each containing 100 mL of the NO_3^- and PO_4^{3-} solutions with specified concentrations. A known amount of the biomass was added to the NO_3^- and PO_4^{3-} solutions, and the flasks were agitated in a shaker. Then, 5 mL of the cell suspension was removed from each flask at different time intervals (0,30, 45 and 60 min) (Amini and Younesi, 2009). The supernatant was separated by centrifugation (10,000 rates per minute (rpm) for 10 min) and used for estimating the dissolved NO_3^- and PO_4^{3-} concentrations. The NO_3^- and PO_4^{3-} concentrations determined by a spectrophotometric methods (Association and Association, 1989)., with a Thermo Scientific UV visible spectrophotometer (300 Evolution, United Kingdom). The NO_3^- and PO_4^{3-} ions uptake capacity was calculated by using Eq. (1):

$$q = \frac{V(C_i - C_e)}{S} \tag{1}$$

where q = ions uptake at equilibrium in mgg^{-1}; C_i = initial ions concentration in mgL^{-1}; C_e = equilibrium NO_3^- and PO_4^{3-} ions concentration in mgL^{-1}; V = volume of solution in L; and S = the dosage of *D. Salina* in the solution in gL^{-1} (El-Naas et al., 2007).

The NO_3^- and PO_4^{3-} ions removal efficiency was defined as the ratio of the concentration of the adsorbed NO_3^- and PO_4^{3-} ions at equilibrium to that of the initial ions in solution. It was calculated using Eq. (2):

$$R = \frac{C_0 - C_e}{C_0} \times 100 \tag{2}$$

where C_0 and Ce = concentrations of the NO_3^- and PO_4^{3-} ions in initial and equilibrium solutions in mgL^{-1}, respectively.

2.4. Desorption and reusing algae biomass

In order to desorb NO_3^- and PO_4^{3-} ions -loaded biomass, HCl (1 M, eluting agent), was used and algae was incubated in the environmental shaker under the same conditions as in the adsorption studies. The *D. salina* was reused for three cycles to determine its reusability.

2.5. The effect of interference of other ions on adsorption process

An industrial application of adsorption process must deal with the fact that the waste streams also contain other ions that may interfere with the uptake of the NO_3^- and PO_4^{3-} ions. These experiments were conducted to evaluate the effect of other interference cations and anions including, $AgSO_4$, $MgSO_4$, K_2SO_4 with the same anion and NaCl, $NaSO_4$, $NaCO_3$ with the same cation on the adsorption of NO_3^- and PO_4^{3-} ions by microalgae *D. salina*. Experimental runs were carried out with 0.05 gL^{-1} biomass dosage with a pH of 7 and 350 mgL^{-1} NO_3^- and PO_4^{3-} concentrations (optimum conditions according to experiments of adsorptions).

2.6. Fourier transform infrared spectroscopy studies

The FTIR spectra of *D. salina* absorbent before and after NO_3^- and PO_4^{3-} adsorption (synthetic and real wastewater) was recorded with NICOLET iS10 Spectrophotometer using solid pellet potassium bromide (KBr) after completely drying the sample at 60 °C up to constant weight. The spectra were taken in the range of 400–4000 cm^{-1}. The dry algal biomass (about 0.1 g) mixed with KBr (0.1 g) and pressed into a tablet form and then FTIR spectrum was recorded.

3. Results and discussion

The history of the commercial use of algae for biological wastewater treatment goes back to 75 years ago first with genus *Chlorella.* In recent years much attention has been paid to use different new species, because ionization of surface functional groups and the binding sites present on the cell wall of algae are important for nutrient uptake. In the present study, the value of microalga, *D. salina* was examined for using as adsorbent on wastewater nutrient remediation and the impacts of variation of pH solution, nutrients concentrations, and algal biomass dosage on the nutrient uptake capacity and removal efficiency were evaluated.

3.1. pH affecting NO_3^- and PO_4^{3-} adsorption by algae

First of all, in order to find out the optimum pH for the adsorption

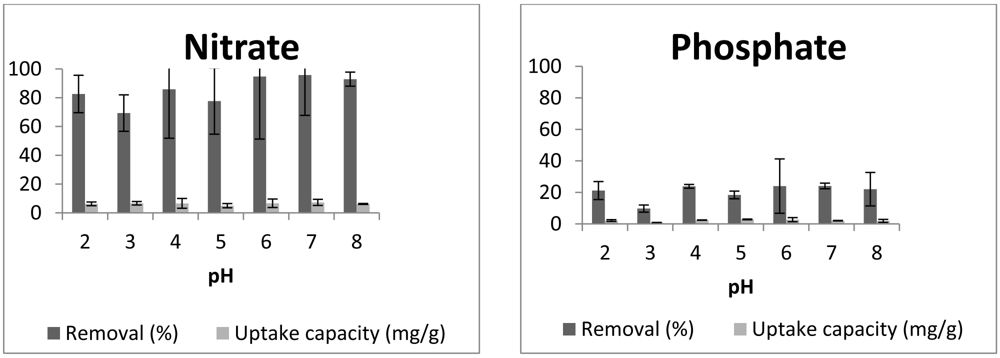

Fig. 1. pH affecting NO_3^- and PO_4^{3-} adsorption by algae.

NO_3^- and PO_4^{3-} by *D. salina*, the solution pH from acidic to alkaline range pH (2–8) was surveyed. The results indicated that the adsorption include uptake capacity and removal efficiency increases with increasing pH from 2 to 7 and reached maximum 7.27 mgg^{-1} uptake capacity with 95.5% removal efficiency for NO_3^- and 2.03 $mg\,g^{-1}$ uptake capacity with 24.0% removal efficiency for PO_4^{3-} at pH,7. The lower removal efficiency by algae was observed at pH 2 (82.6%) to 6 (94.7%) and also pH8 (92.9%) for nitrate. Phosphate removal efficiency also was lower at pH 2 (21.1%) to 6 (23.9%) and 8 (22%) than pH 7, Fig. 1. The nitrate and phosphate uptake capacity has been reduced to above or below pH 7. Therefore, the interaction of the biomass with nitrate and phosphate was favorable at pH 7. This indicates algae biomass can be applied in neutral water (or wastewaters) for the effective removal of NO_3^- and PO_4^{3-} and any deviation on either side of pH 7 would inhibit the uptake rates of ions in the medium.

Solution pH can have a pronounced effect on adsorption since the adsorbent surface charge strongly influence the adsorption of charged ions species (Rashid et al., 2017). This is due to the competitive effect of the H^+ and also due to the fact that the pH affects the ionization of the functional groups on the surface of the adsorbent material (Soumya et al., 2015). At higher pH, more hydroxyl ion exists in the solution which might compete with NO_3^- and PO_4^{3-} for the sorbent site. Also, by increasing pH, the sorbent surface becomes more negative which makes greater repulsion and as a consequence, the adsorption decreases (Rashid et al., 2017). The decrease in the NO_3^- and PO_4^{3-} ions uptake capacity and removal efficiency with the decrease in the pH, may be attributable to destruction of biomass cell wall at low pH (Sharma and Tomar 2008). In summary, the pH study demonstrates that the hydrogen ion concentration affects the complexation reactions or behavior of the different functional groups present in the surface of algal cells as well as to complex formation constants.

Soumya et al. (2015) worked on removal of phosphate and nitrate from aqueous solution using sea grass, *Cymodocea rotundata* beads. Phosphate and nitrate removal by *C. rotundata* beads was observed at pH values ranging 5 to 9. Results of their researches showed that maximum values of removal efficiency (70.9%) for phosphate and (61.8%) for nitrate was obtained when the pH value was 7 and 8 respectively. These results were consistent with the proposed predominant adsorption mechanism that the optimum pH for nutrients removal by the plant tissues should be around 7 at which almost all phosphate exists in the forms of $H_2PO_4^-$ (that is, mononuclear adsorption). If solution pH is higher than the optimum value, poly nuclear interactions may be triggered to consume more adsorption sites. Mallick (2002) worked on a review paper for biotechnological potential of immobilized algae for wastewater N, P and metal removal and in a part of this paper (Mallick, 2002), Vilchez and Vega (1994) were studied the nitrite uptake from wastewater as an initial step required to establish optimal conditions for the potential use of the system in bioreactors. Their results showed that when an initial cell loading of about 30–40 μg chlorophyll g^{-1} gel was used for immobilization of *Chlamydomonas reinhardtii,* the resulting cell beads showed an optimum nitrite uptake rate of 14 μmol nitrite mg^{-1} Chl. h^{-1} at 30 °C and pH of 7.5. This value of pH for optimum conditions of nitrate removal from wastewater is similar to our paper (Vílchez and Vega, 1994). In other paper, Doria et al. (2012) worked on *Scenedesmus acutus* strain of algae for bioremediation of urban wastewater and results indicated that in pH 7, nitrate removal efficiency was obtained 100% as Kang et al. (2006) investigated by the green alga *Haematococcus pluvialis* in primary treated wastewater for simultaneous N and P uptake and in pH value 7.5, nitrate removal was reported 100%. Also Ren and Ogden (2014) studies with microalgae, *Nannochloropsis gaditana* for nitrate removal and results indicated that in pH 7, 40% removal efficiency was obtained. All of these researches are confirms with our study because we obtained maximum removal efficiency 95.78% and 7.27 $mg\,g^{-1}$ uptake capacity of nitrate in pH 7 and also maximum removal efficiency 24.07% and 2.03 $mg\,g^{-1}$ uptake capacity of phosphate was reported in pH 7 (Kang et al., 2006; Doria et al., 2012; Ren and Ogden, 2014). Sabeti et al. (2018) worked on enhanced removal of nitrate and phosphate from wastewater by *C. vulgaris* and according to results maximum removal efficiency with *C. vulgaris* for nitrate was higher than 95% in pH 7.5 and for phosphate was 100% in pH 9 (Sabeti et al., 2018). These results are very similar with our research for nitrate removal. The highest percentage of removal in the present work has been obtained in the near-neutral pH range (7). This pH value is very suitable for removing pollutant substances because it doesn't the need to change the usual conditions of water.

3.2. Biomass dosage affecting NO_3^- and PO_4^{3-} adsorption by algae

The different dosages of algae biomass 0.05, 0.15, 0.25, 0.5, 0.75, 1, 1.5 and 2 $g\,L^{-1}$ were used for adsorption experiments and results are presented in Fig. 2. The amount of $NO_3^-$and PO_4^{3-}ions removed from a solution phase was dependent on the algae biomass dosage and with increasing biomass dosage, NO_3^- and PO_4^{3-} ions uptake per gram of biomass was reduces. With using 0.05 $g\,L^{-1}$ of biomass, the nitrate and phosphate adsorption capacity was 39 and 29.7 $mg\,g^{-1}$, respectively. By increasing the consumption of algae biomass up to 2 $g\,L^{-1}$, these values were decreased to 0.5 and 1.5 $mg\,g^{-1}$ for nitrate and phosphate. In addition, the highest NO_3^- removal efficiency from aqueous solution was found at 0.05 $g\,L^{-1}$ (50%) and more biomass provides a partial aggregation of biomass, which resulted decrease of effective surface area for the adsorption. The using 2 $g\,L^{-1}$ of algae resulted in only 29% removal efficiency. In case of PO_4^{3-} ions, adding more biomass of algae increased removal efficiency up to maximum 73.9% at 2 $g\,L^{-1}$ dosage of algae. Nevertheless, in the next experiments, the optimum biomass consumption was considered 0.05 $g\,L^{-1}$ for removal of both nutrients. Indeed, the adsorption capacity index has a higher relative importance compared to removal efficiency for determining the capability of a

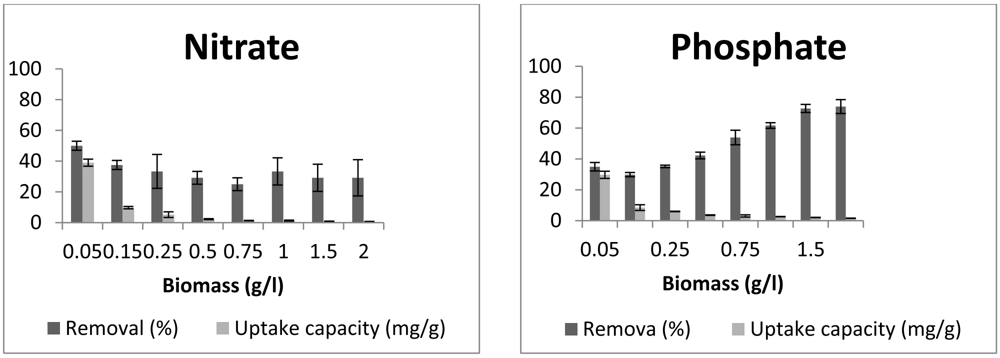

Fig. 2. Biomass dosage affecting NO_3^- and PO_4^{3-} adsorption by algae.

suitable adsorbent and this parameter for PO_4^{3-} was higher in 0.05 g L^{-1} biomass dosage. Therefore, 0.05 g L^{-1} was selected as the optimum biomass dosage for further experiments. The maximum adsorption efficiency at the lowest biomass dosage indicated that electrostatic interactions between cells have a significant negative effect on NO_3^- and PO_4^{3-} ions uptake by algal biomass (Soumya et al., 2015). The high algal biomass dosage may promote self-aggregation which causes the functional group not to bind with ions and also with increasing biomass dosage reduced efficiency in nutrients removal and caused cell leakage. In low values of biomass dosage, the removal efficiency is rapidly increased due to the rate of adsorption site. In fact, with the decrease in adsorbent amount, adsorption sites become profuse compared to nutrients current in the solution (Mehta and Gaur, 2005).

In general, the adsorption increases with decreased in biomass dosages in solutions as agreed by some earlier workers (Soumya et al., 2015). worked on removal of phosphate and nitrate from aqueous solution using sea grass *C. rotundata* beads and for phosphate the maximum removal efficiency was 70.9% when biomass dosages was 0.1 g. Whereas, the phosphate removal at 0.5 and 1 g biomass dosage were recorded to be 60.1% and 45.9%, respectively. In the case of nitrate, minimum removal (47.5%) was recorded at 1 g and maximum removal (61.8%) was observed at 0.1 g. Therefore, similar to our study, maximum removal efficiency was reported in minimum values of biomass dosage. Also Bhat et al. (2008) were investigated on adsorption of metal ions from aqueous medium onto *Catenella repens*, a red alga. Adsorption process was performed for the following biomass concentrations, 0.1, 0.5, 1, 1.5 and 2 gl^{-1}. The qe was found to decrease concomitantly with the increments in biomass concentration and the highest value of qe (259 ± 6.6 mgg^{-1}) was observed at the biomass concentration of 0.25 gl^{-1}. On further increasing the biomass concentration to 2 gl^{-1}, the decrease in qe was happen (Bhat et al., 2008). In other work, Bhainsa and D'Souza. (2009) worked on metal ions adsorption by *Aspergillus fumigates* and varying biomass concentration in the range of 0.3–10 gL^{-1} was used and the results indicated that optimum value of biomass concentration was reported 1 gl^{-1} (Bhainsa and D'Souza, 2009). Likewise (Mehta and Gaur, 2005), used of algae for removing heavy metal ions from wastewater in review paper and showed that in many researches; the amount of ions recovered from a solution was decreased by biomass concentration increasing. For example Roy et al. (1993) for Cd removal with biomass of *Chlorella minutissima* (Roy et al., 1993), Mehta and Gaur (2001) for Cu and Ni adsorption by *C. vulgaris* (Mehta and Gaur, 2001), Gong et al. (2005) for Pb adsorption capacity of *Spirulina maxima* (Gong et al., 2005), Hamdy (2000) for adsorption of Cr, Co, Ni, Cu and Cd by four different algae (Hamdy, 2000), in all of them is expressed that increase in biomass concentration reduces metal adsorption per gram of biomass and therefore decrease in uptake capacity and removal efficiency was happen.

3.3. Initial NO_3^- and PO_4^{3-} concentrations affecting adsorption process by algae

In these experiments, initial nitrate NO_3^- and PO_4^{3-} concentrations from 50 to 350 mg L^{-1} were tested. It was seen that both, uptake capacity and removal efficiency increases with an increase in initial NO_3^- and PO_4^{3-} concentrations. The maximum nitrate and phosphate uptake capacity were 332.5 mgg^{-1} and 544.6 mgg^{-1} respectively, which obtained at 350 mg $L^{-1}$of initial ion concentration. Also the maximum nitrate and phosphate removal efficiency were 54.2% and 82.8% respectively, reached at 350 mg L^{-1} (Fig. 3).

This clearly shows that with increase in ions concentrations in solutions, the removal efficiency by algae increased. As well, it is indicated that high concentrations of nutrients ions had no undesirable effect on the algal cells. Therefore, biomass of *D. salina* could use for polluted solutions and wastewaters including high concentrations of nutrients. Similarly, in other experiment *Neochloris oleoabundans* has

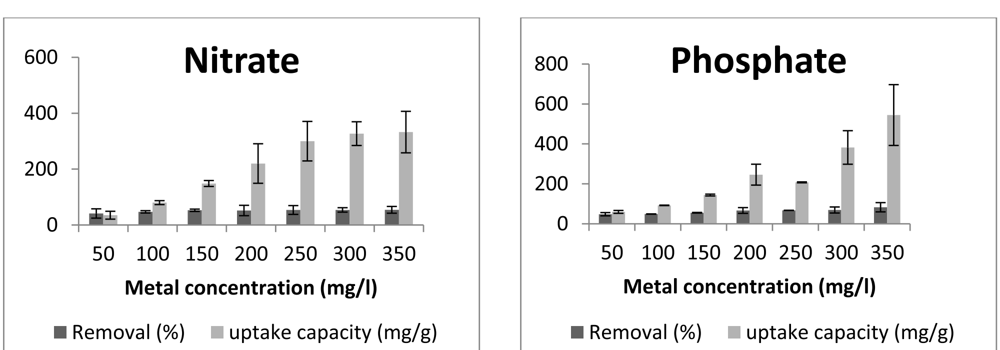

Fig. 3. Initial NO_3^- and PO_4^{3-} concentrations affecting adsorption process by algae.

Table 1
The interference of other anions and cations affecting NO_3^- and PO_4^{3-} adsorption by algae.

	R of PO_4^{3-} (%)	q of PO_4^{3-} (mg g-1)	SD (R)	SD (q)
optimum conditions	82.81152589	544.61	23.1448	152.2118
AgSO4	43.88888889	110.6	3.731952	9.40452
MgSO4	20	75.6	0.523783	1.979899
K2SO4	66.03325416	194.6	20.65894	60.88189
NaCl	58.56353591	222.6	38.41556	146.0176
NaSO4	63.25823224	255.5	27.94113	112.8542
NaCO3	38.81188119	137.2	16.80254	59.39697
	R of NO3- (%)	**q of NO3- (mg g-1)**	**SD(R)**	**SD (q)**
optimum conditions	54.28571429	332.5	12.12183	74.24621
AgSO4	33.33333333	140	11.78511	49.49747
MgSO4	16.04395604	73	0.466224	2.12132
K_2SO4	35.71428571	175	5.050763	24.74874
NaCl	40	490	6.060915	74.24621
NaSO4	37.14285714	195	4.04061	21.2132
NaCO3	28.57142857	120	6.73435	28.28427

been used by Wang and Lan (2011) for remove nitrate. Their data showed that, with increasing initial nitrate concentration, increased nitrate uptake rates and reached to maximum of 1.82 mg L^{-1} h^{-1} at 140 mg NO_3^- and further increase in nitrate concentration to 218 mgL^{-1} reduced nitrate uptake rates. Variation in initial nitrate concentrations can have different effects on nitrate removal efficiency and algal species can grow on low to high concentrations of nitrate and phosphate. Therefore, different strains have their own acute sensitivity to high concentrations of these ions (Wang and Lan, 2011).

Other authors investigated on nitrate removal efficiency with biomaterials and results of all works indicated that high values of removal efficiency (higher than 90%) were obtained in initial ion concentrations of nitrate about 40–200 mg L^{-1}. For example Ergas and Rheinheimer (2004) worked on Drinking water denitrification using a membrane bioreactor and with initial nitrate concentration 200 mg L^{-1}, removal efficiency in effluent of system was 99% (Ergas and Rheinheimer, 2004). In another study, Darbil et al. (2002) worked on batch system on nitrate removal from potable water and according to results of experiments, initial nitrate concentration was variable from 94 mg L^{-1} to very lower values and removal efficiency was obtained 95% and higher values (Darbi et al., 2002). Other researchers include Zhou et al. (2011), Zhao et al. (2011), Liessens et al. (1993) and also Mohseni Bandpi and Elliott (1998) investigated on nitrate removal efficiency and results were 95% with initial concentration 100 mg L^{-1}, 97% with initial concentration 50 mg L^{-1}, 96% with initial concentration 80 mg L^{-1} and 98% with initial concentration 40 mg L^{-1}, respectively (Liessens et al., 1993; Mohseni-Bandpi and Elliott, 1998; Zhao et al., 2011; Zhou et al., 2011). Therefore biomaterials and Microorganisms are being able to treatment of wastewater from nutrient materials in high values. This point is very desirable for wastewaters purification with high amounts of pollutants.

The results of our study for removal percentage (82%) and uptake capacity (544 mg g^{-1}) of phosphate were very high value in initial phosphate concentration 350 mg L^{-1}. Results of other researchers are similar to our study for treatment of phosphate. For example (Soumya et al., 2015) worked on removal of phosphate from wastewater by *C. vulgaris* and their results indicated that the maximum removal percentage of phosphate was 70.9% in 0.1 micro mol. Results of our paper is very desirable and therefore we can use algae *D. salina* for treatment of much polluted wastewaters of nutrients materials.

3.4. The interference of other anions and cations affecting NO_3^- and PO_4^{3-} adsorption by algae

However, nutrients are the most important water contaminant in the world, because of their high water solubility, there are also other type, combinations, and concentrations of anions and cations in wastewaters. Therefore, the presence of multiple ions in municipal wastewaters isa common situation rather than relatively simple and single ion in solutions. So, in the real situation remove NO_3^- and PO_4^{3-} from contaminated water are more complicated and need to investigate close to reality. Despite investigation of single ion in solutions being routinely surveyed in the research literature, in current study, the possibility of removal of $NO_3^-$and PO_4^{3-} were studied in the presence of several other anions and cations. At first step, the presence of salts with different cations and similar anions in the NO_3^- and PO_4^{3-} adsorption solution was investigated to determine the effect of cations on the adsorption process. Therefore, $AgSO_4$, $MgSO_4$, K_2SO_4 salts were used for this experiments. Results showed that, all three salts had inhibitory effects on adsorption capacity and removal efficiency of NO_3^- and PO_4^{3-}. In the next step, salts with different anions and similar cations were examined to determine the effect of the anions on the adsorption process. NaCl, $NaSO_4$ and $NaCO_3$ were used in this step and the observations indicate a negative effect of the presence of anions on the adsorption process, except for NaCl, which significantly increased the uptake capacity of phosphorus from 332 mg g^{-1} to 490 mg g^{-1}. This phenomenon may have occurred due to increased electrical conductivity in the solution and helps to properly position phosphorus molecule in empty cell walls. Results from the analysis of the competition ions effect are shown in Table 1. In multi-nutrients ions systems, other ions compete for binding to algal ligands and the presence of some cations and anions significantly influence the uptake capacity and removal efficiency of other ions by algal cells.

In groundwater and wastewater, anions such as sulfate, nitrate, chloride and carbonate often co-exist along with nutrients. Rashid et al. (2017) worked on effective removal of phosphate from aqueous solution and so the effect of coexisting ions was also studied by separately adding of each of the anions into the reaction mixtures containing phosphate. Results from the analysis of the competition ions (Chloride, Nitrate, sulfate and Carbonate) indicated that, except carbonate, no noticeable effect in the adsorption efficiency was observed due to the presence of any other anions in the solution. The addition of carbonate (CO_3^{2-}) made the overall phosphate solution alkaline by changing the pH from the initial 6.6 to 10.1. This higher pH might be the reason for the decrease of phosphate uptake in this study (Rashid et al., 2017). Maybe in our study, negative effect of anions and cations on removal efficiency and uptake capacity being affected by decreasing in pH value after adding these ions because the hydrogen ion concentration (pH) is one of the most critical controlling parameters in adsorption process (Soumya et al., 2015). Therefore with change pH in solution overall adding anion or cation, impressive decreasing in uptake capacity and removal efficiency were observed.

At presence of NaCl, similar to our study in the media of *Dunaliella* ponds, only a few micro-organisms have the ability to thrive including halotolerant and halophilic bacteria, a few ciliates, a few amoebae, *Artemia salina* and certain fungi (Post et al., 1983; Butinar et al., 2005; Hosseini Tafreshi and Shariati, 2009). Therefore positive effect of NaCl to uptake capacity and removal efficiency of PO_4^{3-} is very desirable and can be applied in wastewater treatment.

3.5. Desorption and reuse of D. salina for NO_3^- and PO_4^{3-} adsorption by algae

In this study, 1 M of HCl was used as eluting agent, for desorption process and reused algae biomass for adsorption experiments. Results of three times desorption and reuses are shown in Table 2. According to these results, the ruse of algae biomass has no significant effect on

Table 2
Desorption and reuse affecting NO_3^- and PO_4^{3-} adsorption by algae.

	q (mg g-1) of NO3-	q (mg g-1) of PO_4^{3-}
optimum conditions	332.5	544.61
After first desorption	234	518
After second desorption	175	429.34
After third desorption	93.36	154

Table 3
Main characteristics of influent wastewater to refinery.

Component	Content, mg/l
pH	7.35
Oil & grease	277
Total solids (TS)	3770
Total suspended solids (TSS)	160
Volatile suspended solids (VSS)	110
Total dissolved solids (TDS)	3610
Total BOD (TBOD)	350
$N-NO_3^-$	118.8
$P-PO_4^{3-}$	13.6

nitrate removal efficiency. But in the case of phosphate, reuse of algae even up to the fourth time desorption also has favorable results and can be used in the process of wastewater treatment. NO_3^- and PO_4^{3-} are both the nutrients that in excessive amounts pollute water resources. However, nitrate control is more difficult because even N_2 from air may also enter water resources. Consequently, the best way is to control the entry of phosphate into water resources. By using algal biomass to remove phosphate and, consequently, changing the ratio of nitrate to phosphate prevention of algal blooms is possible. Therefore, using *D. salina* for the removal of nutrient contamination is a good option. Parab et al. (2005) worked on uranium removal from aqueous solution by coir pith and used HCl for desorption and reuse biomass in adsorption experiments. They were investigated recovery percentage of adsorbed ions 0.01, 0.1, 0.15 and 0.2 concentration (M) of HCl. Results indicate that maximum removal efficiency was obtained in 0.2 M solution of HCl as 99% (Parab et al., 2005). In our study removal efficiency is low in next times of reuse but uptake capacity of biomass of *D. salina* was very high even after three time reuse (93.36 mg g^{-1} for NO_3^- and 154 mg g^{-1} for PO_4^{3-}). Therefore is desirable for use of this biomass in wastewater treatment process. In another work Genç et al. (2003) used HCl for desorption and reuse biomass of fungal *(Trametes versicolor* and *Phanerochaete chrysosporium)* and results showed that the carboxymethyl cellulose – fungus beads could be regenerated using 10 mM HCl and uptake capacity of uranium with these fungal strains after the fourth recovery was 155 mg g^{-1} for *P. chrysosporium* and 305 mg g^{-1} for *T. versicolor* (Genç et al., 2003). These results are desirable and similar to our study. Therefore use of HCl for desorption and reuse of algal biomass is appropriate option in wastewater treatment process (see Table 3).

3.6. Fourier Transform Infrared Spectroscopy Studies for NO_3^- and PO_4^{3-} adsorption by algae

The FTIR technique was applied for the characterization of microalgae absorbent before and after the adsorption of NO_3^- and PO_4^{3-} ions (Fig. 4). Based on FTIR results, the possible functional groups involved in nutrients adsorption using this alga (*D. salina*) were amino, carboxylic, hydroxyl and carbonyl groups. In general, the FT-IR spectra of all the algae preparations have intense peaks at a frequency level of 1076 cm^{-1} representing C—O groups, 1240 cm^{-1} representing P—O groups, 1400 cm^{-1} representing O═C—O stretching of carboxylate groups, 1300, 1470 and 2800 cm^{-1}representing C—H groups, 1540 and 1650 cm^{-1} representing N—H stretching of amino groups and also 3200 cm^{-1} representing *O*—H stretching of hydroxyl groups (Lin et al., 2005; Amini et al., 2012).

After nutrients adsorption, four changes of the functional groups on the adsorbent biomass were detected from the spectrum: decrease of the peak at the region 1000 cm^{-1} and change of carbonyl groups (C—O) on the biomass. Decreasing of the peak at 1400 cm^{-1}, which was carboxylate functional group (O═C—O), and changes of hydroxyl groups (C—H) in 1470 cm^{-1} and also at the regions 1550 and 1650 cm^{-1} decrease in amino groups (N—H) were detected. The overall spectral analysis strongly supports the major role of carboxyl, carbonyl, hydroxyl and amino groups in nutrients binding by the algae biomass (Mehta and Gaur, 2005; Kazy et al., 2009).

In this experiment, the NO_3^- and PO_4^{3-} nutrient solutions were obtained by dissolving corresponding salts called KNO_3 and K_2HPO_4 in distilled water. In the FTIR spectra, KNO_3 has intense peaks at a frequency level of 1386 cm^{-1} representing O═C—O stretching of carboxylate groups (Deng et al., 2017) and 1540 cm^{-1} representing N—H stretching of amino groups (Lee et al., 2018) and also K_2HPO_4 has intense peaks at a frequency level of 1068 cm^{-1} representing C—O groups (Zhang et al., 2018). Therefore, these peaks and their displacement are related to changes in the structure of algae along with the addition of KNO_3 and K_2HPO_4 to solutions and changes in other peaks may imply the functional groups of algae structure that may have undergone significant changes due to the presence of nutrients in solutions and has no significant relations with the structure of added salts to experimental samples.

3.7. Test of optimum conditions for NO_3^- and PO_4^{3-} adsorption by algae in real wastewater

After testing synthetic wastewater in batch conditions and finding optimal removal conditions of nitrate and phosphate, real wastewater which is a mixture of nitrate and phosphate and other materials was studied. The presence of other substances may have generally negative effects or, in some cases, have a positive effect on the adsorption process.

The optimum conditions in the previous steps were pH 7, the biomass of algae g L^{-1}, 0.05 and the initial concentration of nitrate and phosphate 350 mg L^{-1}. In these conditions, the removal efficiency of nitrate and phosphate were 54% and 82%, respectively. Also, the uptake capacity for nitrate and phosphate were 332 mg g^{-1} 544 mg g^{-1}. In order to investigate the effect of microalgae, *D. salina* in real wastewater conditions, the nitrate and phosphate levels of real wastewater which was recorded 118.8 and 13.6 mg L^{-1} respectively increased up to 350 mg L^{-1} to apply the optimal conditions from the previous step. By adjusting the conditions based on the previous step, the nitrate removal efficiency reached to 20% from 54% and for phosphorus reached to 37% from 82%. Also nitrate and phosphate uptake capacity reached to 128 and 222 mg $g^{-1}$from 322 to 544 mg g^{-1} respectively. As mentioned above, uptake capacity is an important factor in the treatment of water and wastewater systems. Therefore, the algae used in the present study can be used not only in laboratory conditions but also in real urban wastewater treatment. The reduction effectiveness of algae to remove nutrients from wastewater may be due to the large presence of pollutants and their negative effects on the adsorption process. Considering the remarkable and desirable performance of algae in nitrate and phosphate removal from urban wastewater, it is recommended for the higher efficiency of *D. salina* algae in real wastewater treatment, first in the initial step (physical) reduces the suspended solids and in the secondary (biological) treatment stage, reduces the amount of biological oxygen demand (BOD) from the wastewater, and finally the algae can be used in the advanced stage of nutrient elimination to achieve the desired results.

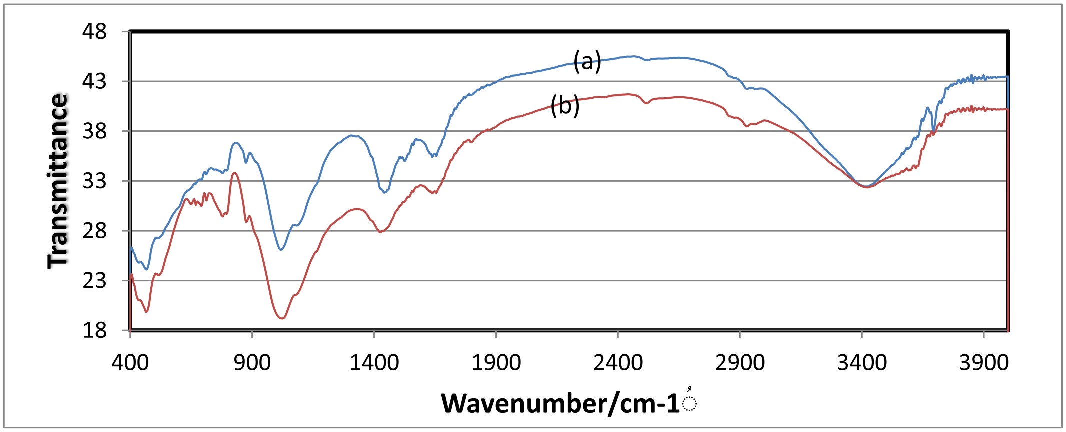

Fig. 4. Fourier Transform Infrared Spectroscopy Studies for NO_3^- and PO_4^{3-} adsorption by algae: (a) before nutrients adsorption; (b) after nutrients adsorption.

4. Conclusion

Excessive accumulation of nutrients discharged into surface water leads to serious ecological problems that affect the health of aquatic organisms, and consequently affect animals and humans health. Therefore, it is necessary to remove these materials to reduce their harmfulness from wastewaters. In this study, green algae, *D. salina* was used in bath experiments to remove nitrate and phosphate from wastewaters and receive to optimum conditions with desirable removal and uptake capacity of these nutrients. The highest adsorption capacity and removal efficiency of nitrate and phosphate were obtained in pH 7, algae biomass, $0.05\, g\, L^{-1}$, and initial concentration of nitrate and phosphate $350\, mg\, L^{-1}$. In these conditions, uptake capacity and removal efficiency of nitrate by *D. salina* were $332\, mg\, g^{-1}$ and 54%, respectively, and uptake capacity and removal efficiency of phosphate were $544\, mg\ g^{-1}$and 82%, respectively. The desorption experiments have also confirmed the effectiveness of this alga in the nutrient removal process. Also presence of other anions and cations had negative effect on NO_3^- and PO_4^{3-} adsorption by algae. Finally in optimum conditions *D. salina* was used for treatment of real wastewater and results reduced than synthetic conditions, but it was still impressive and desirable for use of this alga in wastewater treatments.

Acknowledgment

The authors gratefully acknowledge the generous support of university of Jiroft and also we would like to thank all staff of Iranian Fisheries Science Research Institute (IFSRI), Offshore Research Center, Chabahar, Iran for their valuable help.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101097.

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